PROJECT SUMMARY / ABSTRACT

The long-term goal of the Chodera lab is to develop quantitatively accurate physical modeling techniques that enable the truly rational engineering of novel small molecules to manipulate cellular pathways in desired ways. *This proposal addresses* the current inability of the physical modeling field to account for the dynamic nature of both protein and ligand protonation states, which currently hinders the quantitative accuracy of physical modeling approaches to help guide the synthesis of novel molecules for drug discovery and chemical biology. Neglect of protonation state effects—either the population of a mixture of protonation states or a shift in dominant states upon binding—can lead to errors of several kcal/mol, significant enough to frustrate the ability of physical modeling techniques to prioritize ligands for lead optimization in systems where these effects are present. While protonation state effects in ligand binding have only been studied in detail in a handful of systems, recent evidence suggests that these effects may play an important role in the binding of selective tyrosine kinase inhibitors like imatinib. *The objective of this proposal* is to overcome current limitations in physical modeling approaches by integrating a dynamic treatment of protonation states into quantitatively accurate alchemical binding free energy calculations. We utilize a combined computational-experimental approach to both validate this approach and characterize the pervasiveness of protonation state effects (and the magnitude of errors stemming from their neglect) in the binding of selective kinase inhibitors to their targets of therapy. To accomplish this, we adopt a combined computational-experimental approach. In **Aim 1**, we use fast constant-pH Monte Carlo simulation techniques to identify a set of candidate kinase:inhibitor complexes likely to exhibit significant protonation state effects. In **Aim 2**, we use our unique expertise to develop GPU-accelerated constant-pH alchemical free energy techniques and apply them to characterize the nature and magnitude of protonation-state effects in selective kinase inhibitor recognition. In **Aim 3**, we utilize a variety of experimental techniques—including isothermal titration calorimetry, fluorescence binding affinity measurements, and NMR—to validate the computational predictions of the nature and magnitude of protonation state effects in a set of kinase:inhibitor systems predicted to exhibit significant effects. This approach is *innovative* because it incorporates a dynamic treatment of protonation states into state-of-the-art alchemical binding free energy calculations, using both recent results from nonequilibrium statistical mechanics to drastically boost acceptance rates and optimal multistate reweighting techniques to estimate binding affinities with minimal error. This research is *significant* because it both eliminates a major barrier to the quantitative prediction of protein-ligand binding affinities and establishes the pervasiveness and magnitude of protonation state effects in the binding of selective kinase inhibitors. The research proposed here will ultimately lead to both significant improvements in the quantitative accuracy and domain of applicability of quantitative physical modeling techniques as well as a detailed understanding of the role of protonation state effects in selective kinase inhibitor recognition.